BASALIT-trial: Double blind placebo controlled allergen immunotherapy with rBet v 1-FV in birch related soy allergy

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DOI: https://doi.org/10.1111/all.13112

Received Date : 06-Apr-2016
Revised Date : 14-Nov-2016
Accepted Date : 17-Dec-2016
Article type : Original Article: Gastrointestinal Diseases

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The clinical trial BASALIT (**B**irch **A**ssociated **S**oy **Al**lergy and **I**mmuno-**T**herapy) was funded by the German Federal Ministry of Education and Research (FK 01KG0911).

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Abstract

Background:

Conflicting results exist on the effect of allergen immunotherapy (AIT) on pollen related food allergy. We aimed to investigate the efficacy of one year AIT with the folding variant (FV) of recombinant (r) Bet v 1 on birch related soy allergy.

Methods:

Out of 138 subjects with Bet v 1 sensitization, 82 were positive at double blind placebo controlled food challenge (DBPCFC) with soy. 56/82 were randomized 2:1 (active : placebo). Per protocol population (PPP) had received >150µg of allergen or placebo preparation. Outcome measures: lowest observed adverse effect levels (LOAEL), post interventional occurrence of objective signs (objS) at any dose level, sIgE/IgG4 against Bet v 1 and Gly m 4. Between-group changes were investigated (ANCOVA, Mann-Whitney-U-, Fisher exact test).

Results:

Baseline characteristics including LOAELs were comparable in both groups with objS and subjS occurring in 82% and 95% of active (n=38) versus 78% and 83% of placebo group (n=18). After AIT, objS occurred in 24% and 47%, respectively. LOAEL group differences showed a beneficial tendency (p=0.081) for $LOAEL_{objective}$ in PPP (30 verum, 15 placebo). sIgG4 raised only in active group (Bet v 1: p=0.054, Gly m 4: p=0.037), no relevant changes occurred for sIgE. Only 56% of the intended sample size was recruited.

Conclusion:

For the first time, we present data on the effect of rBet v 1-FV on birch related soy allergy. rBet v 1-FV AIT induced significant immunogenic effects. Clinical assessment showed a tendency in favor of the active group but did not reach statistical significance.

Keywords:

Birch food allergy, soy, allergen specific immunotherapy, recombinant Bet v1, molecular

Frequently used abbreviations

ab	- antibody
AIT	- allergen immunotherapy
ANCOVA	- analysis of covariance
BASALIT	- Birch Associated Soy Allergy and Immuno-Therapy

CM - challenge meal

DBPCFC- Double blind placebo controlled food challenge

FA - food allergy

FAS - Full analysis set

HASR - Histamine adjusted soy reaction

IQR - Interquartile range

LOAEL - Lowest observed adverse effect level

PPP - Per protocol population

QoL - Quality of life

SCIT - Subcutaneous immunotherapy

sIgE/G - specific immunoglobulin E/G

Introduction

Sensitization to major birch pollen allergen Bet v 1 is often associated with pollen related food allergy (FA) [1] and birch related soy allergy became of growing importance during the last years [2-4]. The immunologic basis for birch pollen related FA results from IgE antibodies (ab) that are raised against major pollen allergens but that can also recognize homologous allergens belonging to the pathogenesis-related protein family 10, for example Gly m 4 in soybean [5-7].

There is still controversy as to whether pollen related FA can be successfully treated with allergen immunotherapy (AIT) with pollen allergens [8-11]. As birch pollen AIT was shown to induce Bet v 1 specific (s) IgG4-ab that cross react with related food allergens and inhibit IgE binding by epitope competition, clinical effects of AIT on pollen related FA are to be expected [8,12].

Within previous investigations, birch pollen AIT with either sublingual (SLIT) or subcutaneous (SCIT) application, improved pollen related FA in some studies [13-17] while others could not confirm this [18-20]. The studies that reported improvement [13-17] were not placebo-controlled and did not include DBPCFC for endpoint assessment. The only existing randomized placebo-controlled trial investigated clinical effects of birch pollen AIT (single maximum dose 12.3µg Bet v 1/ml [21]) on birch related hazelnut FA by DBPCFC and was not able to detect group differences [19]. It was suggested, that doses to induce an appreciable effect of birch pollen AIT on pollen related FA should be higher than those sufficient to improve pollen related respiratory symptoms [16, 18, 21].

Because of the growing number of affected patients and increasingly frequent reports on severe reactions [2-4,6], we decided to investigate the effect of rBet v 1-FV AIT (single maximum dose 80µg/ml of Bet v 1/ml [22,23]) on birch related soy FA. The multicentric setting required previous standardization of DBPCFC with soy [24].

Methods

Setting and patient selection

Between 1/2010 and 2/2013, 195 adults (18 – 65 years) with history of birch pollen allergy were recruited in 16 centres (15 German, one Swiss). They underwent standardized allergy interview, skin prick test (SPT) as well as serum IgE test (see fig. 1). We defined eligibility for DBPCFC if patients had positive SPT for birch, ≥ 3.5kU/l of sIgE for birch allergen Bet v 1 and ≥ 0.7kU/l for its soy homologue Gly m 4 (ThermoFisher, Freiburg, Germany). The trial (EudraCT-Nr.: 2009-011737-27) was approved by the competent authorities in Germany and Switzerland; the leading ethic committee was located at University Medical Centre Leipzig (UMCL). All patients provided written informed consent. Main exclusion criteria were pregnancy and unstable asthma. The following drugs were contraindicated during AIT: Ongoing immunosuppression, long-term treatment with tranquilizers/psycho active drugs, betablocker, ongoing/previous anti-IgE therapy. The following drugs were allowed if needed: antihistamines, topical glucocorticosteroids, systemic glucocorticosteroids up to 7.5mg prednisolone equivalent ≤ 5 days. A sample size of 97 subjects (with randomization ratio 2:1) was regarded sufficient to reach a study power of ≥ 90%, based on lowest observed adverse effect levels for objective signs ($LOAEL_{obj}$) and subjective symptoms ($LOAEL_{subj}$) as two primary endpoints without hierarchy. LOAELs (including their heterogeneity) as presented previously [4] were used for calculation of sample size, as well as clinically relevant group differences of 20g in $LOAEL_{obj}$, 10g in $LOAEL_{subj}$ and 15% non-compliance/drop-out rate. NCSS/ PASS software was applied.

Skin prick test

At baseline, SPT was performed with commercially available supplement free soy drinks (single prick to prick test, ALPRO, Uelzena, Germany) and with a panel of respiratory allergens including birch (Allergopharma GmbH & Co KG, Reinbek, Germany) for inclusion (cutoff >/=3mm). Due to unexpected market withdrawal of the drink planned to be applied, only in case of comparable drinks used both before and following AIT, any reduction of wheal diameter at the end of treatment (as binary outcome) was analysed. Also, circle-approximated soy induced wheal area was divided by that induced by histamine (histamine adjusted soy reaction/HASR) in analogy to Dreborg [25].

Double blind placebo controlled food challenge

Standardization of DBPCFC including composition of CM with consistent Gly m 4 levels was previously described [24, 26]. Single dose levels of soy protein were 0.0004 - 0.0044 - 0.05 - 0.15 - 0.5 - 1.5 - 2.5 – 5 - 15g with a maximum cumulative dose of 24.7g protein. Investigators monitored 10 objective signs and patients recorded 8 subjective symptoms on 10cm visual analogue scales (VAS) each. DBPCFC was stopped after dose level 9 or if the patient showed any objective symptoms before.

DBPCFC was defined positive if a patient presented at least one of the following reactions: >/=1 objective sign and/or >/=1 subjective symptoms (VAS value reaching 1.5cm or more and/or two or more subjective symptoms with single VAS values each reaching 0.5cm or more and summing up to 4cm or more in total) [24].

LOAELs corresponding to minimal reactive/threshold dose for objective signs and subjective symptoms were determined. In order to be able to detect any post interventional increase in symptom eliciting doses, we only included patients for AIT in whom DBPCFC was judged as positive at dose level 7 (single dose 2.5g, cumulative 4.7g soy protein) or below. Post interventional DBPCFC was performed within three months after end of AIT.

Laboratory investigations

sIgE- and sIgG4-ab were determined by using ImmunoCAP (Thermo Fisher Scientific, Freiburg, Germany). At screening, sIgE-ab against Bet v 1 and Gly m 4 were measured for inclusion. sIgE- and sIgG4-ab [27] against birch allergens Bet v 1, Bet v 2, soy extract, the soy allergens Gly m 4, Gly m 5, Gly m 6, frequent birch related food allergens Api g 1 (celery), Cor a 1 (hazelnut), Dau c 1 (carrot; made of equimolar ratio of Dau c 1.0104 and Dau c 1.0201), Mal d 1 (apple) and Pru av 1 (cherry) as well as sIgE against cross reacting carbohydrate determinants (MUXF3) were investigated at different time (t) points: t1 - at first injection, t2 - at end of up dosing, t3 at start of maintenance therapy, t4 at fourth dose, and t5 at last injection. Blood samples of all randomized subjects were shipped to Paul-Ehrlich-Institut, Langen, Germany, and stored at -80°C until assayed.

Investigational medical product and therapy

The recombinant hypoallergenic derivative of the major birch pollen allergen rBet v 1-FV as well as the comparative placebo compound (both containing aluminium hydroxide, phenol, sodium chloride, sodium hydrogen carbonate) were manufactured by Allergopharma GmbH & Co. KG (Reinbek, Germany) [22]. Patients were randomized to either active (rBet v 1-FV) or placebo intervention. Up-dosing of patients

started in 2010, 2011 or 2012 between July and October. The starting dose (strength A) was 0.75µg (0.15ml), continued with increasing dosages of 1.5/3.0/5µg (0.3/0.6/1.0ml) and then of 10/20/40/80µg (0.1/0.2/0.4/0.8 ml of strength B). During up-dosing injections were administered at one weekly interval, with a maximum single dose of 80 µg. During prolongation, there were gradually increased injection intervals from 7-28 days. During maintenance, injections were applied every 28 days with a 50% reduction during the birch pollen season. Asthma patients were monitored with peak flow at each trial injection. Patients of per protocol set population (PPP) had received a cumulative dose of at least 150µg major allergen (or placebo equivalent).

Outcome measures

Primary endpoints were DBPCFC-based LOAELs $LOAEL_{obj}$ and $LOAEL_{subj}$. Additionally, occurrence of any objective signs or subjective symptoms at post interventional DBPCFC was investigated. Secondary endpoints were (i) courses of sIgE- and sIgG4-ab levels for Bet v 1, Gly m 4 and other birch related foods from baseline to end of treatment, (ii) pre-post changes of reactivity at SPT (if suitable) as well as (iii) pre-post changes of FA related quality of life (QoL) via FA quality of life questionnaire – adult form (FAQLQ-AF) [28,29].

Statistics

The confirmatory analysis was based on the full analysis set (FAS) with baseline LOAEL instead of missing values at end of study if applicable and intention-to-treat principle. We focused on two primary endpoints without hierarchy - both $LOAEL_{obj}$ and $LOAEL_{subj}$ since it was unclear in advance how many patients would react with objective signs at DBPCFC. Post-interventional measures after the ingestion of soy-containing CM were used in nonparametric analysis of covariance (ANCOVA) with corresponding baseline measures as covariates [30]. The global significance level of the clinical trial was limited to $\alpha=5\%$. The test-wise α-levels were adjusted for multiplicity according to the Bonferroni-Holm method [31] based on the ordered p values observed. The smaller observed p-values $p(1)$ (corresponding with either $LOAEL_{obj}$ or $LOAEL_{subj}$) had to be lower than/equal to $\alpha(1)=\alpha_{global}/2=0.025\geq p(1)$ to identify a significant treatment effect in at least one LOAEL. For the 2nd comparison $p(2)\leq\alpha(2)=0.05$ should be observed to establish significance in both endpoints. SAS macros developed and provided by the University of Göttingen within the German Research Foundation (DFG) sponsored project "Ordinal Data" were used to compare the treatments without any further covariates in confirmatory analysis. In a planned sensitivity analysis, the same procedures as in confirmatory analysis were applied within the per protocol population (PPP). Secondary and safety outcomes were analyzed by Fishers exact test, repeated-measures ANCOVA, and Mann-Whitney U test, with neither adjustment for multiplicity nor missing value imputations.

Results

Characteristics of screening and study population

195 patients (63.4% female, mean (SD) of age 38.1(12.8)) years were screened. 138 patients were eligible for DBPCFC, 82 (59.4%) had positive DBPCFC at baseline [24]. Out of those 82, 56 patients were randomized (2:1) to interventional AIT with rBet v 1-FV (n=38) or placebo (n=18) (figure 1). 19/38 (50%) and 8/18 (44%) had a history of previous reactions to any soy product. 13/38 (34%) and 8/18 (44%) underwent a previous AIT. Table 1 contains major characteristics of the trial population. 54/56 randomised patients started the intervention, meaning that only 56% (54/97) of the intended sample size could be recruited within the given time frame. Major protocol violations occurred in 9/54 subjects: in the active group, two subjects did not fulfil criteria for positive DBPCFC and in three other cumulative AIT allergen doses applied were below 150µg. In four subjects of active and two from placebo group no post interventional DBPCFC was performed. PPP included 45 subjects (for details see table 1).

Allergen immunotherapy and adverse events

Maintenance phase was reached in 31/37 (84%) patients of active and in 16/17 (94%) of placebo group. Cumulative allergen doses are given in table 1. During treatment course, 119 injection related adverse events (AE) occurred in 22/37 (60%) patients of the active and in 9/17 (53%) of the placebo group. AEs were almost exclusively mild: 64/119 (54%), consisted in localized injection site reactions, 13/119 (11%) were skin reactions with generalized urticaria in one subject, 15/119 (13%) had respiratory (nose/lung; 3 asthmatic responses in 2 patients), 7/119 (6%) eye and 20/119 (17%) unspecific symptoms. There were no injection related serious AEs. During AIT, systemic intake of antihistamines was documented in 21/54 (39%) and of short-term systemic glucocorticosteroids in 8/54 (15%) subjects (due to skin lesions in n=6 or asthma in n=2).

Double blind placebo controlled food challenges

At baseline, objective signs were present in 45/56 (80%): blistering/swelling of oral mucosa 47% (21/45), flush 18%, urticaria 2% angioedema 7%, conjunctivitis 18%, rhinitis 18%, peakflow reduction 9%, heart rate increase 9%, drop of blood pressure 2%, gastrointestinal symptoms 4%.Subjective symptoms occurred in 51/56 (91%, most frequently reported were oral tingling/blistering 34% (19/56, dysphagia 23% and itching 14%.Nausea, abdominal pain and dizziness occurred in 7%, respectively, dyspnea in 4% and perceived lip swelling in 2%. Cumulative doses at occurrence of first symptoms and signs are shown in figure 2 and DBPCFC-based outcome measures listedin table 2. No relevant dysbalances were seen between both groups at baseline with regard to

LOAELs, type of objective signs or type of subjective symptoms.

In confirmatory analyses, in PPP (but not in FAS), $LOAEL_{obj}$ tended to be higher in the active group (treated with rBet v 1-FV) compared with the placebo group (p=0.081). Individual dose level changes are shown in figure 3. With the best/worst case groups-related observations (regarding between-group differences, heterogeneity and $LOAEL_{obj}$ as exclusive primary endpoint) we calculated that between 81 and 162 patients (best/worst case scenario) would have been necessary to provide significant test results. A post interventional increase of one dose level or more occurred in 20/26 (77%) subjects of active and in 9/14 (64%) subjects of placebo group who presented with objective signs both at baseline and post intervention.

Skin prick test

Maximum histamine induced wheal diameter at baseline was 5.0 mm [Interquartile range/IQR 4.0;6.0] in active and 5.0 mm [3.9;5.4] in placebo group, with soy 6.8 mm [5.0;9.0] vs. 7.0 mm [3.9;8.6] and with birch 9.5 mm [7.0;10.5] vs. 8.0 mm [6.9;9.6], respectively. In the randomised population, the same type of soy drink at baseline and at control was used in 43/54 (80%) of subjects, and in PPP, in 26/30 (87%) of active and in 14/15 (93%) of placebo group. In PPP, there was a non-significant (p=0.116) shift toward reduced soy induced wheal diameter in active (6.0 mm) compared with placebo group (7.3 mm) at postinterventional SPT. HASR changed from 3.27 fold area (SD 4.48) at baseline to 1,57 fold (1.1) post intervention in the active, and from 2.33 fold (1.46) to 2.89 fold (3.71) in the placebo group, respectively. No significant between-group differences were found, although a descriptive change toward smaller HASR were observed within the rBet v 1-group compared to unchanged reactivity in the placebo group.

Laboratory investigations

At baseline, sIgE-ab > 0.35kU/l against Cor a 1, Dau c1, Mal d1, Pru av1 were detected in all subjects, against Api g 1 in 51/54, against soybean extract in 10/54, Bet v 2 in 5/54 and MUXF3 in 4/54. Low level Gly m 5 and Gly m 6 sIgE was seen exclusively in one patient (0.52 and 0.99kU/l, respectively). At baseline (t1), no relevant group differences were shown for any sIgE-ab investigated. In neither group, courses of sIgE-ab showed significant differences from t1 to t5 for Bet v 1, Gly m 4 or any other allergen investigated.

In the active, but not in the placebo group, a significant increase from t1 to t5 for sIgG4-ab against Bet v 1 (figure 4a,b), Gly m 4 (figure 4c), Cor a 1 (figure 4d), Api g 1 (p<0.0001; respectively), Dau c1 (p<0.001), Mal d 1 (p>0.006) and Pru av 1 (p<0.015) was found. When comparing post interventional t5 values, there was a significant increase of sIgG4-ab against Gly m 4 (p=0.037) and Cor a 1 (p=0.033)

in the active group; borderline significant increases occurred for sIgG4-ab against Bet v 1 (p=0.054), Bet v 2 (p=0.074), Pru av 1 (p=0.088) and Mal d 1 (p=0.06). No significant differences were seen for IgG4-ab against Gly m 5, Gly m 6, soybean extract, CCD, Api g1, Dau c 1.

Quality of life

FAQLQ-AF scores at baseline are given in table 1. No significant differences between active and placebo group were found with nearly unchanged post interventional scores.

Discussion

The BASALIT trial is the first randomized, double blind placebo controlled trial evaluating the efficacy of a component based birch pollen AIT on birch related soy FA in a multicentre setting. The immunologic basis for this type of FA results from Bet v 1 homologous soy allergen Gly m 4 which shows 53% amino acid sequence identity and 63% sequence peptide similarity to Bet v 1 [5,6].

Subjects with combined Bet v 1 and Gly m 4 sensitization were included. Even though IgE values cannot safely predict reactivity at DBPCFC [32, 33] we initially decided to only include patients with defined IgE cut off values. The rBet v 1-FV extract was chosen for AIT as it shows low IgE-binding [22,23,34], was well tolerated in higher doses with no increase in adverse effects compared with the native extract and induced a significant increase in birch pollen sIgG4-ab [22,23,35].

A main limiting factor for investigations on FA is the availability of standardized CM with standardized allergen content to be used within DBPCFC. For this trial we set up standardized soy CM with consistent levels for protein contents and Gly m 4 levels [24,26]. Also, a standardized evaluation system was established [24]. Despite of intensified efforts, we only reached 56% of the intended sample size within the given time frame.

The rBet v 1-FV extract was well tolerated and, as in previous trials [22, 35], the safety profile was comparable with placebo; no rBet v 1-FV related severe adverse effects occurred. Subjects of active group had received cumulative Bet v 1 allergen doses being eight to twenty fold higher compared with previous AIT studies on pollen related FA (i.e. 50µg [18] and 150µg [12]).

$LOAEL_{obj}$ at post interventional DBPCFC tended to be higher in active compared with placebo group in per protocol population. In detail, at baseline DBPCFC objective signs occurred in 82% of active versus 78% of placebo group and at post interventional DBPCFC in only 23% versus 47%. No significant differences were seen with regard to $LOAEL_{subj}$ or any overall occurrence of subjective symptoms between groups. Possibly, effects could have been more pronounced by applying higher

soy allergen doses, as only 80% of all subjects presented with objective signs at baseline DBPCFC. Allergen doses were comparable to those having been reported in a previous trial, where, however, another food matrix had been applied [4].

In the active group, some subjects showed post interventional decrease of LOAEL while, remarkably, in the placebo group, a LOAEL increase was not rare (figure 3). One might speculate that there is a fluctuating course of the pollen related FA being influenced not only by birch pollen season but also by other clinical cofactors that might not have been fully eliminated at some time points of DBPCFC.

In vitro investigations indicated an immunogenic effect of the rBet v 1-FV extract as there was a significant increase of Bet v 1 sIgG4-ab in active group which was more than twice as high as in previous trials using birch allergen extracts with lower Bet v 1 allergen levels [12,15]. Despite a significant increase of sIgG4-ab to all Bet v 1 homologues investigated we noticed significant between-group effects only for sIgG4-ab against Gly m 4 and Cor a 1. However, sIgG4-ab against Bet v 1, Bet v 2, Pru av 1 and Mal d 1 tended to be higher in rBet v 1-FV group.

In accordance with our data, an increase of sIgG4-ab against Bet v 1 and Cor a 1 was demonstrated in another trial investigating the effect of one year whole extract birch pollen-SCIT in birch related hazelnut allergy [19]. Even though sIgG4-ab were expected to be functionally blocking, authors failed to show clinical improvement of hazelnut allergy after one year of treatment [19].

In another recent trial, authors discussed that birch pollen-AIT may induce Bet v 1 sIgG4–ab that cross-react with related food allergens Mal d 1 and Cor a 1 and inhibit sIgE binding by epitope competition [12]. This effect was detectable after one year but was even stronger after three years of AIT (cumulative allergen dose 500µg) [12], and reduction in food allergen reactive sIgE-ab started only later than 12 months of AIT. Interestingly, sIgG4-ab were not simply IgE epitope identical as more than 35% of predicted IgE epitopes of Bet v 1, Mal d 1 and Cor a 1 were not recognized by sIgG4-ab. It was concluded that SCIT induced sIgG4-ab may not cover all IgE specificities [12].

Due to unexpected market withdrawal, the soy drink being scheduled for baseline and control SPT was only available in a subgroup of patients. Therefore, we were not able to finally assess the influence of AIT on soy induced wheal diameter or on histamine adjusted wheal area [25, 36].

Finally, in our trial, a rather small impact of birch related FA on QoL was measured which confirmed a previous single center experience [29].

In summary, for the first time we present data on the effect of AIT with the major birch pollen

allergen Bet v 1 on birch pollen related FA to soy. This is the only study having investigated AIT induced changes in FA symptoms by means of standardized food challenge procedures and CM with consistent soy allergen levels [24,26]. One year SCIT with rBet v 1-FV showed a clear immunogenic effect on sIgE- and sIgG4-ab against Bet v 1 and Bet v 1 homologous food allergens. Clinical assessment showed a tendency in favor of the active group but did not reach statistical significance. One reason may be that the treatment period was too short to induce changes on relevant antibody levels, on epitopes or on clinical symptoms. Another reason lies in the fact that we failed to recruit the intended study sample size and, therefore, conclusive clinical results could not be reached.

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Aknowledgement:

This trial was funded by the German Federal Ministry of Education and Research (FK 01KG0911). Trial medication as well as skin prick test solutions were kindly supplied by Allergopharma GmbH & Co. KG (Reinbek, Germany). We thank Uelzena (Uelzen, Germany) for kindly providing Supro® soy protein, Dr. R. Frontini and J. Zimmer (Pharmacy of the University Hospital, Leipzig, Germany) for production and Dr. rer. nat. A. Willenberg (Institute of laboratory medicine, Clinical chemistry and Molecular diagnostics, University Hospital, Leipzig, Germany) for microbiological investigations of challenge meals, the ZKS (Leipzig, Germany) for study logistics, data management, monitoring and biometry as well as B. Flokstra-de Blok (Groningen, The Netherlands) for kindly providing FAQL-questionnaire. Our thanks also go to the members of the Data and Safety Monitoring Board (Prof. Dr. Vera Mahler, Universität Erlangen, Germany; Prof. Dr. T. Jakob, Universität Freiburg, Germany; Prof. Dr. Oliver Kuss, Universität Düsseldorf, Germany) and to those of the Food allergy interest group of the German Society for Allergy and Immunology (DGAKI) for stimulating discussions. The Department of Dermatology at the RWTH (Prof. Dr. H. Merk), Aachen, Germany, screened two patients who were not eligible for DBPCFC. Ms. St. Randow (Paul-Ehrlich-Institut, Langen, Germany) is acknowledged for excellent technical assistance in Gly m 4 quantification and ImmunoCAP measurements. Dr. Lothar Vogel (Paul-Ehrlich-Institut, Langen) is acknowledged for ImmunoCAP data transfer and summary. ThermoFisher (Freiburg, Germany and Uppsala, Sweden) kindly provided non-commercial Pru av 1 and Dau c 1 ImmunoCAPs (prepared by Dr. Jonas Lidholm, Uppsala, Sweden) and liquid reagents.

Author contributions:

RT, AF, AS, JCS generated the concept of the study including set up of challenge meal and defined standardized procedures for DBPCFC; SV, MW, TW, JKT, BBW gave advice to DBPCFC and preparation of challenge meal; TH did analysis of Gly m 4 levels in challenge meals and organised and supervised sIgE and sIgG4; RT, JCS, MW, TB, TW, UJ, BBW, RB, AK, JS, HB, JKT, FR, JR, JS, KS recruited patients for DBPCFC and SCIT. All authors revised and approved the manuscript.

Conflicts of interest:

RT report unrestricted research grant from ALK-Abello, fees for lectures and advisory boards from ALK-Abello, Novartis, fees for lectures from Meda, Shire, travel grants from Shire, all outside the submitted work. AF and AS report a grant from Funding Program for clinical trials of German Ministry for education and research (BMBF), BMBF/ DLR Funding-ID 01 KG 0911, for conduct of the study but

without institutional or personal conflicts of interest. BBW received consulting honoraria from Phadia ThermoFisher. JS reports grants from ALK-Abello, Sanofi, Pfizer, MSD, Novartis and personal fees from Roche (outside the submitted work). UJ reports grants from Federal Ministry for Education and Research (BMBF) during the conduct of the study and public Research grants from Deutsche Forschungsgemeinschaft, from Federal Ministry of Economy and Technology and from industry (Chiesi; Allergopharma GmbH & Co. KG, outside the submitted work). RB reports grants, clinical trial fees, fees for lectures and advisory board from ALK-Abello, Allergopharma, Bencard, Leti, Novartis, Stallergenes and fees for lectures from HAL. FR has worked as adviser for ALK Abelló Arzneimittel GmbH, Bencard, DST GmbH and Dr. Gerhard Mann chem.-pharm. Fabrik GmbH and has received speaker´s honorarium from ALK Abelló Arzneimittel GmbH, Astra Zeneca, Bencard, Novartis Pharma GmbH, HAL, MEDA Pharma GmbH & Co, Semperit Technische Produkte Gesellschaft m.b.H, and Stallergenes GmbH (outside the submitted work). TH reports personal fees (payment for organising lectures) from Akademie Fresenius and non-financial support (travel/accommodation/meeting expenses unrelated to activities listed) from European Academy of Allergology and Clinical Immunology, from Deutscher Allergie- und Asthmabund e.V. and from International Life Science Institute/Health and Environmental Sciences Institute. SV reports personal fees from Food Allergy Resource and Research Program, Lincoln, NE USA, personal fees from Medical University of Vienna, Austria, grants from Monsanto Company, personal fees from American Academy of Asthma, Allergy and Immunology, personal fees from Deutsche Dermatologische Gesellschaft, personal fees from Westdeutsche Arbeitsgemeinschaft für pädiatrische Pneumologie und Allergologie e.V., Koln, Germany, personal fees from Gesellschaft für pädiatrische Allergologie und Umweltmedizin, personal fees from Ärzteverband Deutscher Allergologen, personal fees from Swiss Society for Allergy and Immunology, personal fees from Schattauer Allergologie Handbuch, personal fees from Elsevier Nahrungsmittelallergien und Intoleranzen, personal fees from Karger Food Allergy: Molecular Basis and Clinical Practice, non-financial support from German Research Foundation, non-financial support from Federal Institute for Risk Assessment, nonfinancial support from European Directorate for the Quality of Medicines and Health Care, non-financial support from European Academy of Allergy and Clinical Immunology, non-financial support from Deutscher Allergie- und Asthmabund, non-financial support from Association Monegasque pour le Perfectionnement des Connaissances des Médicins, non-financial support from Federal Office of Consumer Protection and Food Safety, non-financial support from German Chemical Society (GDCh), non-financial support from AKM Allergiekongress, non-financial support from International Union of Immunological Societies, outside the submitted work. Other authors declare no conflicts of interest.

Table 1

	Active (rBet v 1-FV) group		Placebo group	
	Randomized	PPP	Randomized	PPP
N (randomized/PPP)	38	30	18	15
Sex female	25 (66%)	20 (67%)	12 (67%)	10 (67%)
Age (years) [++]	37.8 (14.6)	38.1 (15.0)	37.4 (13.8)	36.8 (13.6)
BMI (kg/m²)[++]	24.5 (3.7)	24.2 (3.1)	23.8 (3.6)	23.6 (3.9)
SPT birch positive (baseline)	37 (97%)	29 (97%)	18 (100%)	15 (100%)
SPT soy positive (baseline)	36 (95%)	28 (93%)	16 (89%)	14 (93%)
Total IgE-ab (kU/L baseline) [+]	163 [74-375]	153 [74-3]	147 [67-242]	136 [71-220]
Specific IgE-ab Bet v 1 (kU/L; baseline)[+]	34 [17-66]	34 [23-69]	28 [16-64]	30 [16-73]
Specific IgE-ab Gly m 4 (kU/L; baseline)[+]	9 [4-16]	11 [5-16]	6 [4-11]	6 [4-16]
FAQLQ total score (baseline) [+]	3.8 [2.9-5.2]	3.7 [2.8-5.2]	3.8 [2.3-5.1]	3.7 [2.3-5.1]
FAIM score (baseline) [+]	3.2 [2.7-4.2]	3.1 [2.7-4.0]	2.8 [2.3-3.8]	2.7 [2.3-3.3]
Cumulative allergen dose at AIT (µg) [+]	1013 [99-1440]	1031 [930-1120]	1000* [960-1040]	1013* [960-1040]
Number of visits during treatment[+]	21 [20-23]	22 [20-24]	20 [20-22]	21 [20-22]

Table 1: Characteristics of randomized patients/ per protocol population

No significant differences were seen regarding patients' characteristics and interventional courses between both investigational groups in either population (PPP – per protocol population); [++] mean(standard deviation); [+] median[interquartile range/IQR]), BMI – body mass index, AIT – allergen immunotherapy, SPT – skin prick test; *according calculated amount; FAQOL –food allergy quality of life; FAIM – food allergy independent measure

Table 2

	Active (rBet v 1-FV) group	**Placebo group**
Baseline DBPCFC (n)	38	18
Objective signs	82%	78%
$LOAEL_{obj}$	4.7g [0.7-24.7]	2.2g [0.2-9.7]
Subjective symptoms	95%	83%
$LOAEL_{subj}$	2.2g [0.7-4.7]	0.7g [0.2-2.2]
Post interventional DBPCFC (n)	33 (of 37 treated)	15 (of 17 treated)
Objective signs	24%	47%
$LOAEL_{obj}$	24.7g [24.7-24.7]	24.7g [2.2.-24.7]
Subjective symptoms	22/33 (67%)	9/15 (60%)
$LOAEL_{subj}$	4.7 g [0.7-24.7]	2.2g [2.2-24.7]
Post interventional DBPCFC (n) in PPP	30	15
Objective signs	23%	47%
$LOAEL_{obj}$*	24.7g [24.7-24.7]	24.7g [2.2.-24.7]
$LOAEL_{subj}$	7.2 g [0.7-24.7]	2.2g [2.2-24.7]

Table 2: Double blind placebo controlled food challenge (DBPCFC)-related outcome measures

Data presented for both interventional groups (including primary endpoints) with median values [interquartile range] of lowest observed adverse effect levels for objective signs and subjective symptoms ($LOAEL_{obj/subj}$); no significant group differences after SCIT (* in per protocol population [PPP] between-group effect for treatment with p-value =0.081) were observed.

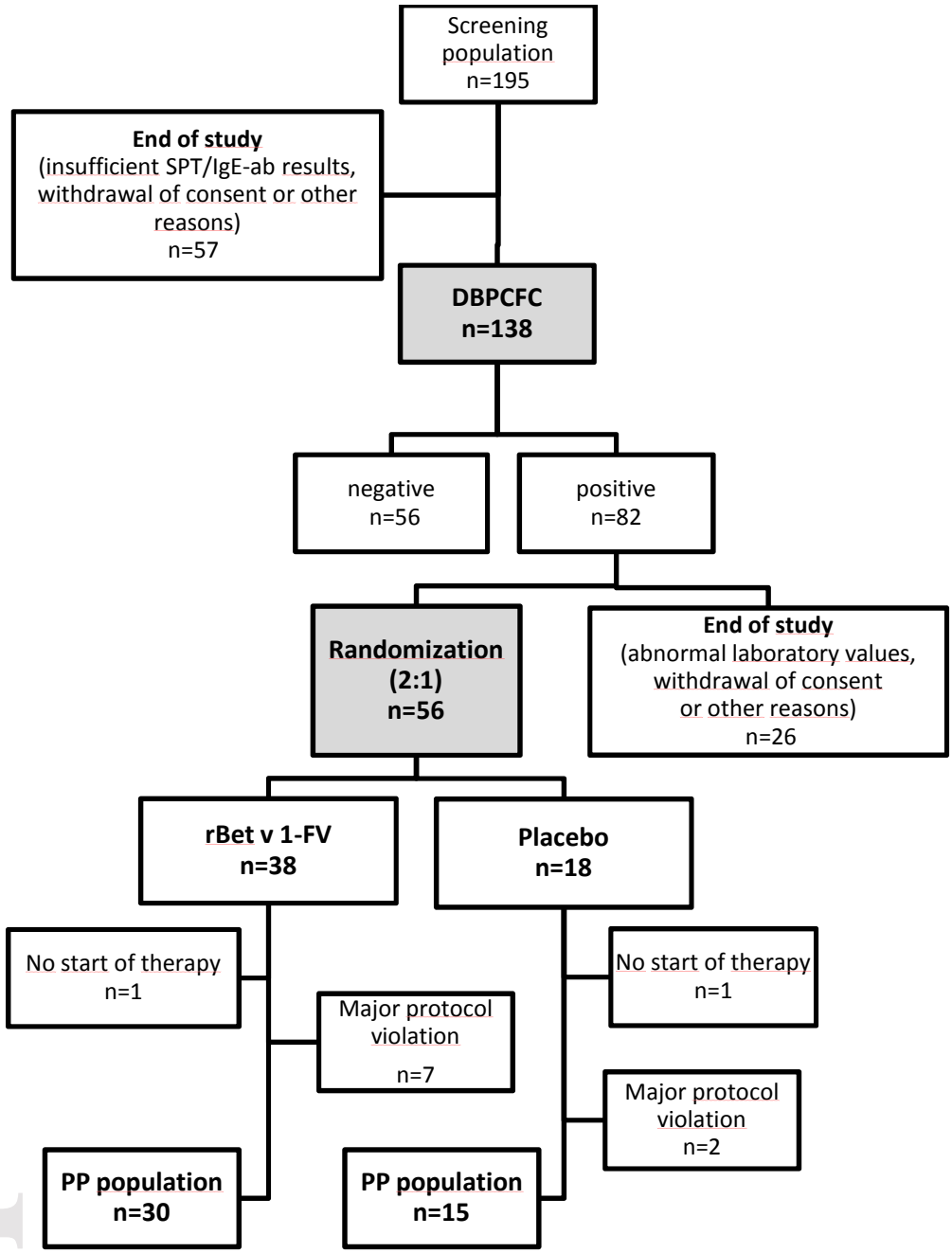

Figure 1

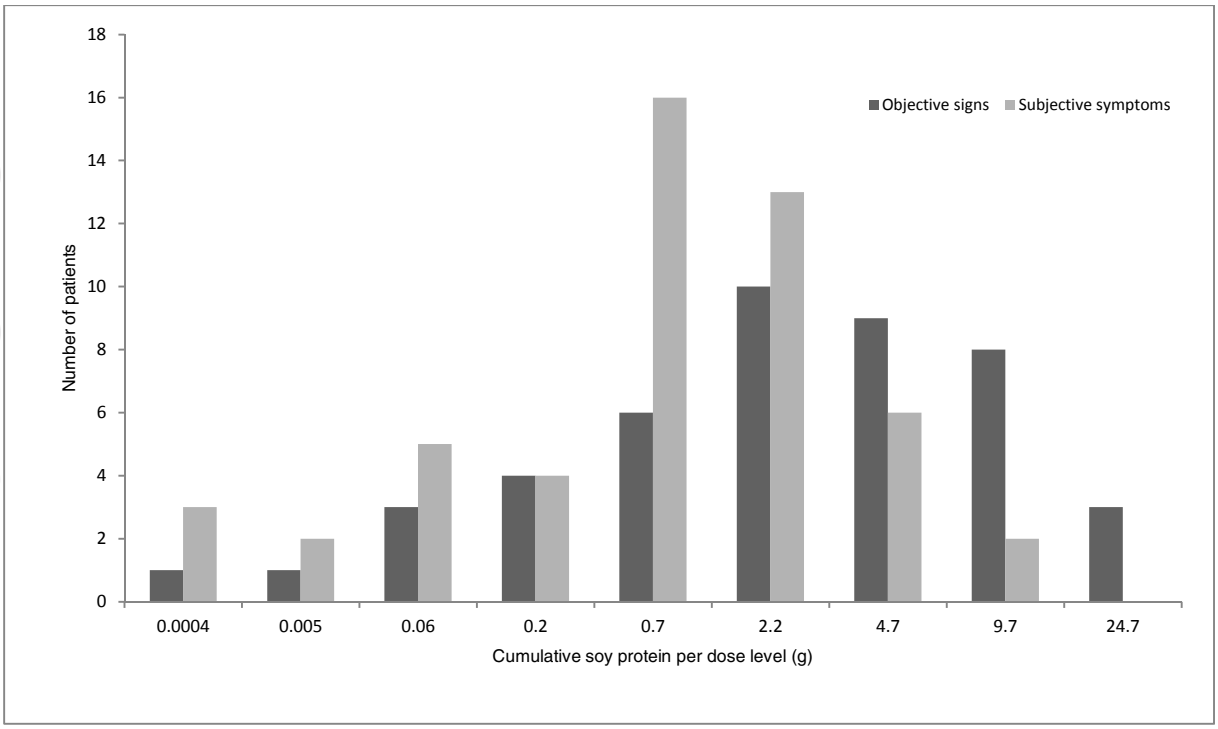

Figure 2

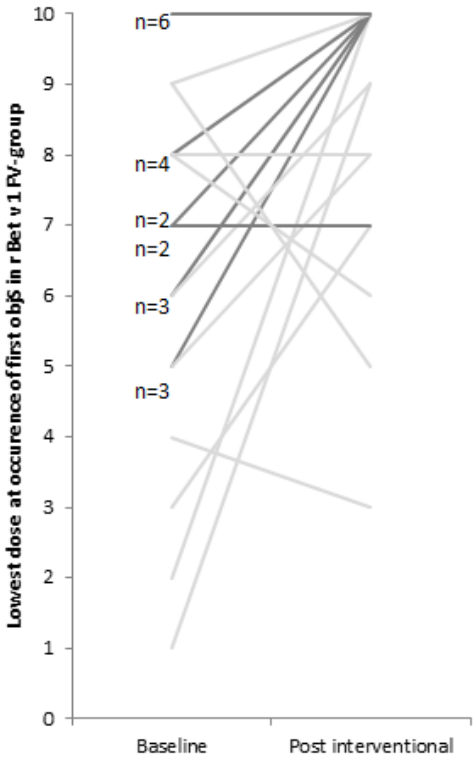

Figure 3 (left)

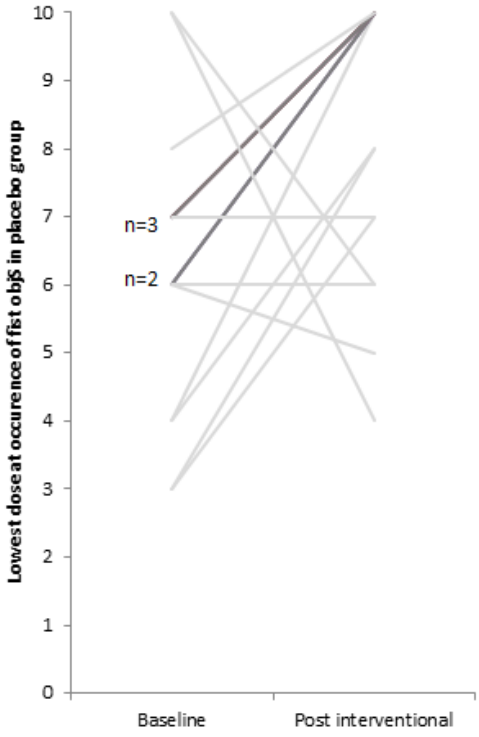

Figure 3 (right)

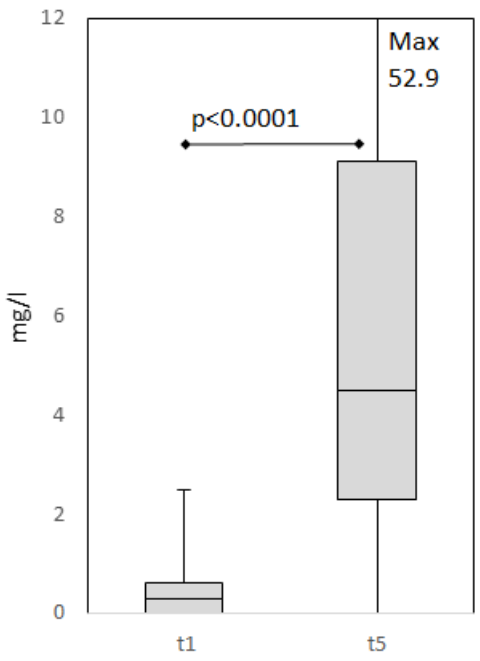

Fig 4a

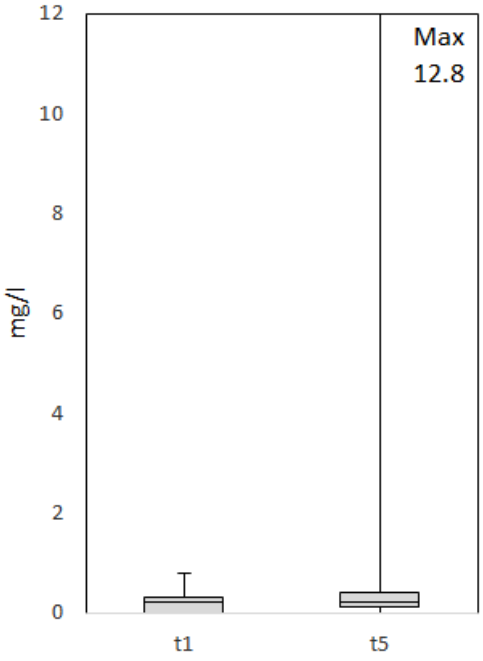

Fig 4b

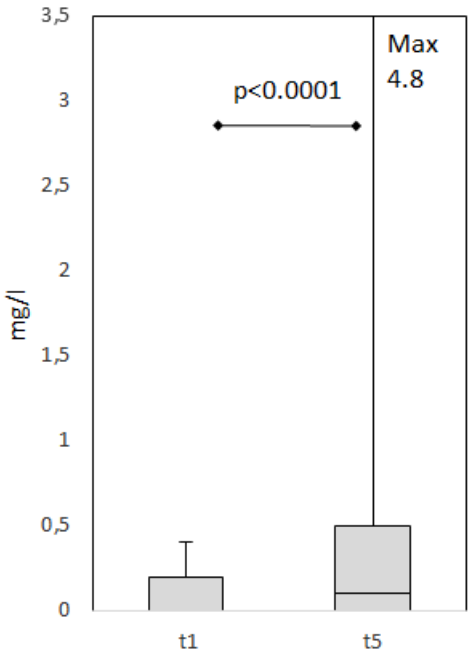

Fig 4c

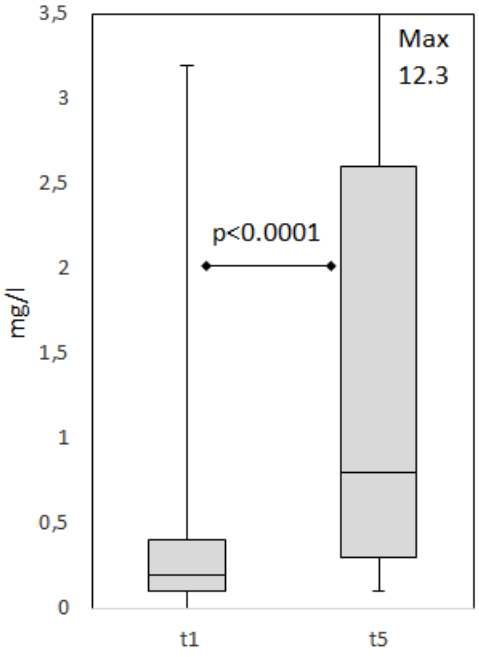

Fig 4d